



## SEQUENCE LISTING

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TECH CENTER 1600/2900

<110> BARCHIESI, Rino  
DEL GIUDICE, Giuseppe  
RAPPUOLI, Rino

<120> DETOXIFIED MUTANTS OF BACTERIAL ADP-RIBOSYLATING TOXINS  
AS PARENTERAL ADJUVANTS

<130> 2302-1393 / PP01393.002

<140> 09/044,696

<141> 1998-03-18

<160> 5

<170> PatentIn Ver. 2.0

<210> 1

<211> 711

<212> DNA

<213> Artificial Sequence

<220>

<221> CDS

<222> (1)..(708)

<220>

<223> Description of Artificial Sequence: wild-type Subunit  
A from E. coli heat labile toxin

<400> 1

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Asn Gly Asp Arg Leu Tyr Arg Ala Asp Ser Arg Pro Pro Asp Glu Ile  
1 5 10 15

aaa cgt ttc cgg agt ctt atg ccc aga ggt aat gag tac ttc gat aga 96  
Lys Arg Phe Arg Ser Leu Met Pro Arg Gly Asn Glu Tyr Phe Asp Arg  
20 25 30

gga act caa atg aat att aat ctt tat gat cac gcg aga gga aca caa 144  
Gly Thr Gln Met Asn Ile Asn Leu Tyr Asp His Ala Arg Gly Thr Gln  
35 40 45

acc ggc ttt gtc aga tat gat gac gga tat gtt tcc act tct ctt agt 192  
Thr Gly Phe Val Arg Tyr Asp Asp Gly Tyr Val Ser Thr Ser Leu Ser  
50 55 60

ttg aga agt gct cac tta gca gga cag tat ata tta tca gga tat tca 240  
Leu Arg Ser Ala His Leu Ala Gly Gln Tyr Ile Leu Ser Gly Tyr Ser  
65 70 75 80

ctt act ata tat atc gtt ata gca aat atg ttt aat gtt aat gat gta 288  
Leu Thr Ile Tyr Ile Val Ile Ala Asn Met Phe Asn Val Asn Asp Val  
85 90 95

att agc gta tac agc cct cac cca tat gaa cag gag gtt tct gcg tta 336  
Ile Ser Val Tyr Ser Pro His Pro Tyr Glu Gln Glu Val Ser Ala Leu

100 105 110  
 ggt gga ata cca tat tct cag ata tat gga tgg tat cgt gtt aat ttt 384  
 Gly Gly Ile Pro Tyr Ser Gln Ile Tyr Gly Trp Tyr Arg Val Asn Phe  
 115 120 125  
 ggt gtg att gat gaa cga tta cat cgt aac agg gaa tat aga gac cgg 432  
 Gly Val Ile Asp Glu Arg Leu His Arg Asn Arg Glu Tyr Arg Asp Arg  
 130 135 140  
 tat tac aga aat ctg aat ata gct ccg gca gag gat ggt tac aga tta 480  
 Tyr Tyr Arg Asn Leu Asn Ile Ala Pro Ala Glu Asp Gly Tyr Arg Leu  
 145 150 155 160  
 gca ggt ttc cca ccg gat cac caa gct tgg aga gaa gaa ccc tgg att 528  
 Ala Gly Phe Pro Pro Asp His Gln Ala Trp Arg Glu Glu Pro Trp Ile  
 165 170 175  
 cat cat gca cca caa ggt tgt gga gat tca tca aga aca atc aca ggt 576  
 His His Ala Pro Gln Gly Cys Gly Asp Ser Ser Arg Thr Ile Thr Gly  
 180 185 190  
 gat act tgt aat gag gag acc cag aat ctg agc aca ata tat ctc agg 624  
 Asp Thr Cys Asn Glu Glu Thr Gln Asn Leu Ser Thr Ile Tyr Leu Arg  
 195 200 205  
 gaa tat caa tca aaa gtt aag agg cag ata ttt tca gac tat cag tca 672  
 Glu Tyr Gln Ser Lys Val Lys Arg Gln Ile Phe Ser Asp Tyr Gln Ser  
 210 215 220  
 gag gtt gac ata tat aac aga att cgg gat gaa tta tga 711  
 Glu Val Asp Ile Tyr Asn Arg Ile Arg Asp Glu Leu  
 225 230 235

<210> 2

<211> 236

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: wild-type Subunit  
A from E. coli heat labile toxin

<400> 2

Asn Gly Asp Arg Leu Tyr Arg Ala Asp Ser Arg Pro Pro Asp Glu Ile  
1 5 10 15

Lys Arg Phe Arg Ser Leu Met Pro Arg Gly Asn Glu Tyr Phe Asp Arg  
20 25 30

Gly Thr Gln Met Asn Ile Asn Leu Tyr Asp His Ala Arg Gly Thr Gln  
35 40 45

Thr Gly Phe Val Arg Tyr Asp Asp Gly Tyr Val Ser Thr Ser Leu Ser  
50 55 60

Leu Arg Ser Ala His Leu Ala Gly Gln Tyr Ile Leu Ser Gly Tyr Ser

65		70		75		80									
Leu	Thr	Ile	Tyr	Ile	Val	Ile	Ala	Asn	Met	Phe	Asn	Val	Asn	Asp	Val
				85					90					95	
Ile	Ser	Val	Tyr	Ser	Pro	His	Pro	Tyr	Glu	Gln	Glu	Val	Ser	Ala	Leu
			100					105					110		
Gly	Gly	Ile	Pro	Tyr	Ser	Gln	Ile	Tyr	Gly	Trp	Tyr	Arg	Val	Asn	Phe
		115					120					125			
Gly	Val	Ile	Asp	Glu	Arg	Leu	His	Arg	Asn	Arg	Glu	Tyr	Arg	Asp	Arg
	130					135					140				
Tyr	Tyr	Arg	Asn	Leu	Asn	Ile	Ala	Pro	Ala	Glu	Asp	Gly	Tyr	Arg	Leu
145				150						155					160
Ala	Gly	Phe	Pro	Pro	Asp	His	Gln	Ala	Trp	Arg	Glu	Glu	Pro	Trp	Ile
			165						170					175	
His	His	Ala	Pro	Gln	Gly	Cys	Gly	Asp	Ser	Ser	Arg	Thr	Ile	Thr	Gly
		180						185					190		
Asp	Thr	Cys	Asn	Glu	Glu	Thr	Gln	Asn	Leu	Ser	Thr	Ile	Tyr	Leu	Arg
	195						200					205			
Glu	Tyr	Gln	Ser	Lys	Val	Lys	Arg	Gln	Ile	Phe	Ser	Asp	Tyr	Gln	Ser
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Glu	Val	Asp	Ile	Tyr	Asn	Arg	Ile	Arg	Asp	Glu	Leu				
225					230					235					

Sub  
E31

<210> 3  
 <211> 723  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <221> CDS  
 <222> (1)..(720)

<220>  
 <223> Description of Artificial Sequence: wild-type CT  
 subunit A

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Asn Asp Asp Lys Leu Tyr Arg Ala Asp Ser Arg Pro Pro Asp Glu Ile	
1 5 10 15	
aag cag tca ggt ggt ctt atg cca aga gga cag agt gag tac ttt gac	96
Lys Gln Ser Gly Gly Leu Met Pro Arg Gly Gln Ser Glu Tyr Phe Asp	
20 25 30	
cga ggt act caa atg aat atc aac ctt tat gat cat gca aga gga act	144
Arg Gly Thr Gln Met Asn Ile Asn Leu Tyr Asp His Ala Arg Gly Thr	
35 40 45	

cag acg gga ttt gtt agg cac gat gat gga tat gtt tcc acc tca att 192  
 Gln Thr Gly Phe Val Arg His Asp Asp Gly Tyr Val Ser Thr Ser Ile  
 50 55 60  
 agt ttg aga agt gcc cac tta gtg ggt caa act ata ttg tct ggt cat 240  
 Ser Leu Arg Ser Ala His Leu Val Gly Gln Thr Ile Leu Ser Gly His  
 65 70 75 80  
 tct act tat tat ata tat gtt ata gcc act gca ccc aac atg ttt aac 288  
 Ser Thr Tyr Tyr Ile Tyr Val Ile Ala Thr Ala Pro Asn Met Phe Asn  
 85 90 95  
 gtt aat gat gta tta ggg gca tac agt cct cat cca gat gaa caa gaa 336  
 Val Asn Asp Val Leu Gly Ala Tyr Ser Pro His Pro Asp Glu Gln Glu  
 100 105 110  
 gtt tct gct tta ggt ggg att cca tac tcc caa ata tat gga tgg tat 384  
 Val Ser Ala Leu Gly Gly Ile Pro Tyr Ser Gln Ile Tyr Gly Trp Tyr  
 115 120 125  
 cga gtt cat ttt ggg ggt ctt gat gaa caa tta cat cgt aat agg ggc 432  
 Arg Val His Phe Gly Val Leu Asp Glu Gln Leu His Arg Asn Arg Gly  
 130 135 140  
 tac aga gat aga tat tac agt aac tta gat att gct cca gca gca gat 480  
 Tyr Arg Asp Arg Tyr Tyr Ser Asn Leu Asp Ile Ala Pro Ala Ala Asp  
 145 150 155 160  
 ggt tat gga ttg gca ggt ttc cct ccg gag cat aga gct tgg agg gaa 528  
 Gly Tyr Gly Leu Ala Gly Phe Pro Pro Glu His Arg Ala Trp Arg Glu  
 165 170 175  
 gag ccg tgg att cat cat gca ccg ccg ggt tgt ggg aat gct cca aga 576  
 Glu Pro Trp Ile His His Ala Pro Pro Gly Cys Gly Asn Ala Pro Arg  
 180 185 190  
 tca tcg atc agt aat act tgc gat gaa aaa acc caa agt cta ggt gta 624  
 Ser Ser Ile Ser Asn Thr Cys Asp Glu Lys Thr Gln Ser Leu Gly Val  
 195 200 205  
 aaa ttc ctt gac gaa tac caa tct aaa gtt aaa aga caa ata ttt tca 672  
 Lys Phe Leu Asp Glu Tyr Gln Ser Lys Val Lys Arg Gln Ile Phe Ser  
 210 215 220  
 ggc tat caa tct gat att gat aca cat aat aga att aag gat gaa tta 720  
 Gly Tyr Gln Ser Asp Ile Asp Thr His Asn Arg Ile Lys Asp Glu Leu  
 225 230 235 240  
 tga 723

<210> 4

<211> 240

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: wild-type CT

subunit A

<400> 4

Asn Asp Asp Lys Leu Tyr Arg Ala Asp Ser Arg Pro Pro Asp Glu Ile  
 1 5 10 15  
 Lys Gln Ser Gly Gly Leu Met Pro Arg Gly Gln Ser Glu Tyr Phe Asp  
 20 25 30  
 Arg Gly Thr Gln Met Asn Ile Asn Leu Tyr Asp His Ala Arg Gly Thr  
 35 40 45  
 Gln Thr Gly Phe Val Arg His Asp Asp Gly Tyr Val Ser Thr Ser Ile  
 50 55 60  
 Ser Leu Arg Ser Ala His Leu Val Gly Gln Thr Ile Leu Ser Gly His  
 65 70 75 80  
 Ser Thr Tyr Tyr Ile Tyr Val Ile Ala Thr Ala Pro Asn Met Phe Asn  
 85 90 95  
 Val Asn Asp Val Leu Gly Ala Tyr Ser Pro His Pro Asp Glu Gln Glu  
 100 105 110  
 Val Ser Ala Leu Gly Gly Ile Pro Tyr Ser Gln Ile Tyr Gly Trp Tyr  
 115 120 125  
 Arg Val His Phe Gly Val Leu Asp Glu Gln Leu His Arg Asn Arg Gly  
 130 135 140  
 Tyr Arg Asp Arg Tyr Tyr Ser Asn Leu Asp Ile Ala Pro Ala Ala Asp  
 145 150 155 160  
 Gly Tyr Gly Leu Ala Gly Phe Pro Pro Glu His Arg Ala Trp Arg Glu  
 165 170 175  
 Glu Pro Trp Ile His His Ala Pro Pro Gly Cys Gly Asn Ala Pro Arg  
 180 185 190  
 Ser Ser Ile Ser Asn Thr Cys Asp Glu Lys Thr Gln Ser Leu Gly Val  
 195 200 205  
 Lys Phe Leu Asp Glu Tyr Gln Ser Lys Val Lys Arg Gln Ile Phe Ser  
 210 215 220  
 Gly Tyr Gln Ser Asp Ile Asp Thr His Asn Arg Ile Lys Asp Glu Leu  
 225 230 235 240

<210> 5

<211> 240

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: wild-type Subunit  
 A from E. coli heat labile toxin

<400> 5

Asn Gly Asp Arg Leu Tyr Arg Ala Asp Ser Arg Pro Pro Asp Glu Ile  
1 5 10 15  
Lys Arg Ser Gly Gly Leu Met Pro Arg Gly His Asn Glu Tyr Phe Asp  
20 25 30  
Arg Gly Thr Gln Met Asn Ile Asn Leu Tyr Asp His Ala Arg Gly Thr  
35 40 45  
Gln Thr Gly Phe Val Arg Tyr Asp Asp Gly Tyr Val Ser Thr Ser Leu  
50 55 60  
Ser Leu Arg Ser Ala His Leu Ala Gly Gln Ser Ile Leu Ser Gly Tyr  
65 70 75 80  
Ser Thr Tyr Tyr Ile Tyr Val Ile Ala Thr Ala Pro Asn Met Phe Asn  
85 90 95  
Val Asn Asp Val Leu Gly Val Tyr Ser Pro His Pro Tyr Glu Gln Glu  
100 105 110  
Val Ser Ala Leu Gly Gly Ile Pro Tyr Ser Gln Ile Tyr Gly Trp Tyr  
115 120 125  
Arg Val Asn Phe Gly Val Ile Asp Glu Arg Leu His Arg Asn Arg Glu  
130 135 140  
Tyr Arg Asp Arg Tyr Tyr Arg Asn Leu Asn Ile Ala Pro Ala Glu Asp  
145 150 155 160  
Gly Tyr Arg Leu Ala Gly Phe Pro Pro Asp His Gln Ala Trp Arg Glu  
165 170 175  
Glu Pro Trp Ile His His Ala Pro Gln Gly Cys Gly Asn Ser Ser Arg  
180 185 190  
Thr Ile Thr Gly Asp Thr Cys Asn Glu Glu Thr Gln Asn Leu Ser Thr  
195 200 205  
Ile Tyr Leu Arg Glu Tyr Gln Ser Lys Val Lys Arg Gln Ile Phe Ser  
210 215 220  
Asp Tyr Gln Ser Glu Val Asp Ile Tyr Asn Arg Ile Arg Asp Glu Leu  
225 230 235 240

Sub  
E21